EXHIBIT A

Nitric Oxide and Restenosis

John P. Cooke MD PhD

A Report for Vascular Architects
September 16, 2002

Nitric Oxide and Restenosis

Restenosis: The Bane of Angioplasty.

Balloon angioplasty is demonstratively effective in the treatment of hemodynamically significant vascular lesions, and is useful in relieving symptoms secondary to obstructive disease. However, the therapeutic utility of angioplasty is limited by re-narrowing of the vessel lumen. Acutely (minutes to days after the procedure), this re-narrowing is due to elastic recoil, and to thrombosis. Chronically(2-6 months), the re-narrowing is due to myointimal hyperplasia as well as adventitial fibrosis and negative remodeling(Fig1). Thrombosis has been reduced by more effective anti-platelet and anti-thrombotic regimens, and elastic recoil and negative remodeling have been eliminated by vascular stents. However, the problem of myointimal hyperplasia remains, and causes significant in-stent restenosis in about 20% of cases. New drug eluting stents may eliminate this remaining problem, and early experience with immunosuppressive agents taxol and sirolimus have been extremely encouraging. Another class of agents which may be equally useful in combination with a drug delivery stent is represented by the NO donors. The following discussion reviews the evidence supporting development of this alternative therapeutic approach.

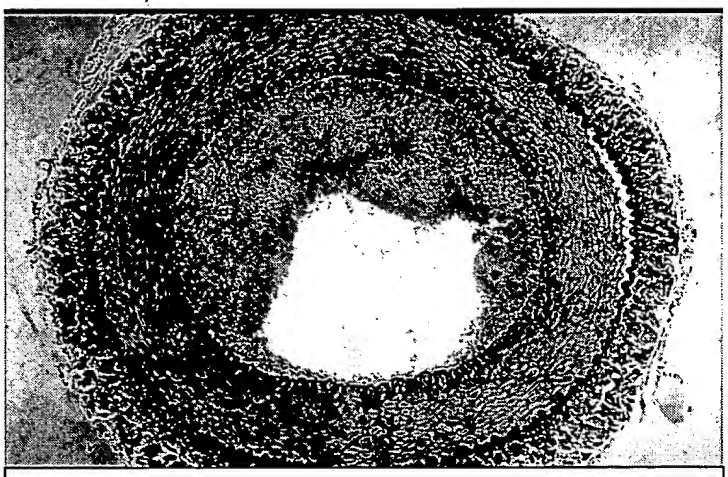

Fig 1. Rabbit iliac artery 4 weeks after angioplasty showing lesion of myointimal hyperplasia.

The Endothelium and Nitric Oxide

In 1998, three American scientists won the Nobel Prize in Medicine or Physiology for their discovery and characterization of a potent endogenous vasodilator elaborated by the endothelium. This endothelium-derived relaxing factor was first described by Robert Furchgott in a citation classic published in 1980(1). Subsequently, Louis Ignarro provided evidence that nitric oxide (NO) was the indentity of this relaxing factor (2). Approximately 10 years before Furchgott's discovery, Ferid Murad had shown that

exogenous nitrovasodilators, such as sodium nitroprusside and nitroglyerin, cause vasodilation by stimulating soluble guanylate cyclase to produce cyclic guanosine monophosphate (cGMP; 3). Ignarro documented that the endothelium derived relaxing factor behaved in the same way. These discoveries had significant ramifications in cardiovascular biology (and in other fields as well).

Endothelium derived NO is derived from the metabolism of L-arginine by NO synthase (NOS). There are three isoforms of NOS: eNOS (the endothelial isoform), nNOS(the neuronal isoform), and iNOS(the inducible isoform, first described in inflammatory cells). These isoforms are not entirely cell specific, eg. eNOS is also found in platelets, and iNOS can be induced in most cells exposed to inflammatory cytokines (4).

In addition to inducing vasodilation, NO elaborated by the endothelium can inhibit platelet adhesion and aggregation; reduce leukocyte adherence and infiltration into the vessel wall; and suppress the proliferation and migration of vascular smooth muscle cells(5). For these reasons, NO has been described as an anti-atherogenic molecule(6).

Nitric oxide and Vascular Structure

Indeed, enhancing the production of vascular NO by administration of the NO precursor (L-arginine) has been shown to inhibit atherosclerosis in mouse and rabbit models(7-9). Furthermore, oral administration of L-arginine has been shown to increase vascular NO synthesis after balloon angioplasty, improve vascular relaxation, and to inhibit restenosis in rat and rabbit models(10-12). Presumably, in this case the NO is derived from iNOS in the injured vessel wall, as angioplasty removes the endothelial source of NOS.

Similar effects have been achieved by transiently transfecting the vessel wall with a plasmid construct encoding eNOS, so as to generate more NO locally. In the rat carotid artery, eNOS gene transfer using lipofection or adenoviral techniqe increased vasular NO elaboration, increased vascular cGMP levels, reduced proliferation of vascular cells, and reduced restenosis after vascular injury (13,14). Similarly, transfection of the injured vessel wall with an adenoviral construct encoding human iNOS inhibits myointimal hyperplasia in rat and pig models of balloon angioplasty. In vivo iNOS gene transfer to injured rat carotid arteries, resulted in a near complete (>95%) reduction in neointima formation even when followed longterm out to 6 weeks post-injury(15). This protective effect was reversed by the continuous administration of an iNOS selective inhibitor L-N6-(1-iminoethyl)-lysine(15). In an animal model more relevant to human vascular healing, iNOS gene transfer (5 x 10(8) PFU/pig) to injured porcine iliac arteries in vivo was also efficacious, reducing intimal hyperplasia by 52%(15). Similar results have been obtained in porcine coronary arteries after angioplasty using the Infiltrator catheter for intramural administration of an adenoviral construct encoding human eNOS (16).

Conversely, genetic or pharmacological inhibition of vascular NO elaboration accelerates atherosclerosis and restenosis. In the hypercholesterolemic NZW rabbit, chronic administration of NOS antagonists increased plaque area and thickness(17). In the eNOS deficient mouse, atherosclerosis is accelerated. When this mouse is bred with the

hypercholesterolemic apo E deficient mouse, atherosclerosis is severe, and even results in atherosclerotic aortic aneurysms(18).

Antagonism of NO synthase by administration of L-NAME exacerbates myointimal hyperplasia after experimental angioplasty (19). To conclude, endogenous NO produced by the vessel wall, causes vasodilation, inhibits platelet and leukocyte adhesion, suppresses cellular proliferation and migration, and prevents vascular lesion formation. Similar observations have been made with exogenous NO donors as described below.

NO donors and Platelet Inhibition

There is a substantial body of data indicating that NO donors suppress platelet adherence to the vessel wall, and inhibit platelet aggregation, at doses that are clinically relevant(20). The effect of NO donors is mediated by activation of soluble guanylate cyclase within the platelet(21). The subsequent production of cGMP leads to the activation of cGMP dependent kinases which phosphorylate proteins such as vasodilator-stimulated phosphoprotein (VASP; 22). In platelets, VASP and VASP phosphorylation have recently been demonstrated to be involved in the inhibition of agonist-induced platelet aggregation and, in particular, integrin $\alpha_{IIb}ß_3$ activation(23). Because platelet aggregation participates in thrombosis at the time of balloon angioplasty, NO-induced platelet inhibition maintains lumen patency immediately after injury. Furthermore, platelets aggregating at the site of vascular injury release growth factors known to activate vascular smooth muscle migration and proliferation such as platelet-derived growth factor (PDGF; 24). Accordingly, by inhibiting platelet adherence and aggregation at the site of vascular injury, NO further inhibits the proliferative vascular response to injury.

NO donors and Leukocyte Infiltration

The mechanism by which NO inhibits monocyte adhesion is probably multifactorial. NO can inhibit monocyte adhesion to the endothelium, mediated by cGMP modulation of adhesion signaling(25). However, NO also downregulates the endothelial expression of monocyte chemotactic protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1), which play critical roles in monocyte-vessel wall interaction(26-28). By contrast, inhibition of NO synthase increases the expression of endothelial proteins required for monocyte adhesion(27). Studies from our laboratory and others implicate the existence of an oxidant-sensitive transcriptional pathway that activates the expression of VCAM-1 and MCP-1(27-29). Endogenous NO or exogenous NO donors, inhibit endothelial elaboration of superoxide anion, reduce the activity of NFκB, suppress the stimulated expression of VCAM-1 and MCP-1, and reduce endothelial adhesiveness for monocytes. NO may exert these effects in part by inhibiting the generation of superoxide anion by oxidative enzymes(30).

NO Donors and Vascular Smooth Muscle Migration and Proliferation

A major component of in-stent restenosis is the migration and proliferation of vascular smooth muscle cells(Fig 2). NO inhibits vascular smooth muscle cell proliferation(31). NO inhibits smooth muscle mitogenesis at distinct points in the cell cycle by cGMP-dependent(late G1 phase) and –independent(S phase) mechanisms(32). In one study,

vascular smooth muscle cells (VSMCs) were transduced with an adenoviral vector encoding eNOS (AdeNOS) or beta-galactosidase (Ad beta Gal). eNOS expression was detected in transduced VSMCs and cGMP levels were increased. These effects were associated with a delay in cell cycle progression and upregulation of p27 and p21(33).

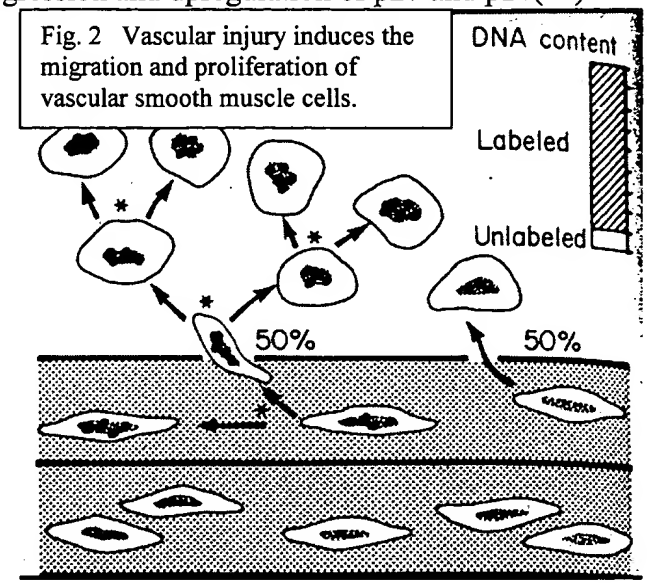

Fig. 2 Vascular injury induces the migration and proliferation of vascular smooth muscle cells.

Similarly, exogenous NO donors inhibit vascular smooth muscle proliferation. DETA/NO, a well-characterized NO donor of low MW (163 Da) with a half-life for NO release of 20 hours suppressed vascular smooth muscle cell proliferation in culture completely but without evidence of toxicity (34).

After vascular injury the migration of VSMCs from the media to the intimal space is an important component of the process of restenosis. The effect of NO donors on this process has been studied in vitro using migration assays. In one such study, the migration of primary cultured VSMCs (derived from canine femoral artery) was assessed and related to cGMP levels(35). The stable analogue of cGMP, 8-Bromo-cGMP, inhibited VSMC migration. When inducible nitric oxide synthase (iNOS) was induced by 24-hour preincubation with lipopolysaccharide and interleuken-1beta, basal migration decreased and cGMP production increased. Under these conditions, insulin further reduced VSMC migration, an effect which was blocked by the nitric oxide synthase inhibitor LNMMA, as well as by 1-H-1[1,2,4]oxadiazolo-[4, 3a]quinoxolin-1-one, a selective inhibitor of guanylate cyclase. These data suggest that NO-induced increases in cGMP reduce VSMC migration. However, there is evidence that NO may elicits opposite effects on cell migration and proliferation in primary versus subcultured cells (36)

NO and Apoptosis

NO induces apoptosis in vascular smooth muscle cells (37). In cultured vascular smooth muscle cells, addition of the NO donor molecules S-nitroso-N-acetylpenicillamine or sodium nitroprusside to VSMC dose-dependently induced apoptosis as documented by DNA laddering and quantified by analysis of cellular chromatin morphology. The mediator role of the guanylate cyclase signaling pathway in NO-induced apoptosis was evidenced by induction of apoptosis by the 8-bromo-cGMP analogue; potentiation of NO-induced apoptosis by cGMP-specific phosphodiesterase inhibition; and prevention of NO-induced apoptosis by inhibition of the cGMP-dependent protein kinase 1 alpha.

By contrast, NO donors have anti-apoptotic effects in cultured endothelial cells (38), suggesting that the effect of NO as a modulator of apoptosis is cell-specific and dependent on the presence of certain cytokines, growth factors, or oxidative stress (39).

A rapidly emerging body of evidence suggests that vascular remodeling and lesion formation are determined in large part by the balance between cell growth and cell death by apoptosis(40,41). NO beneficially modulates both. By suppressing vascular smooth muscle cell proliferation, and by increasing apoptosis of VSMCs as well as infiltrating cells (see below), NO reduces the accumulation of cells in the intimal space. Furthermore, by reducing endothelial cell apoptosis, NO assists in the process of re-endothelialization of the wounded vascular segment.

Endogenous and exogenous NO suppresses the accumulation of inflammatory cells after vascular injury. Immunohistochemical studies document the activation of iNOS in the intimal macrophages and vascular smooth muscle cells of human atherosclerotic plaque(42). In the presence of superoxide anion, which is produced under these conditions, the product of iNOS is quickly transformed into peroxinitrite anion, a highly reactive free radical(43) which itself is cytotoxic and may also induce apoptosis by causing DNA strand fragmentation(44). Both NO or peroxynitrite anion could induce apoptosis of vascular smooth muscle cells(37,41).

Exogenous NO donors can induce apoptosis of inflammatory cells in plaque. In atheromatous aortic segments from hypercholesterolemic NZW rabbits, ex vivo addition of sodium nitroprusside (10uM) to the medium caused a time-dependent increase in apoptosis of vascular cells (largely macrophages) in the intimal lesion(45)

NO Donors and Restenosis

As documented by the in vitro studies discussed above, NO is a pleiotropic molecule with effects on multiple processes involved in restenosis. Accordingly, it is not surprising that in all animal models of vascular injury, the great majority of investigators have found that NO donors administered systemically or locally inhibit restenosis. NO donor molecules of several structural classes reduced intimal thickening in rabbits, pigs, mice, and rats(46-48). Inhaled NO(80 ppm NO for 14 days) inhibited restenosis in rat carotid arteries without causing any hemodynamic changes(49).

An interesting new approach has been to combine NO donors with other agents that may have utility in the immediate or chronic setting of vascular injury. In rat and mouse models of myointimal hyperplasia, oral administration of NO-NSAIDS or NO-aspirin formulations have been shown to inhibit restenosis, whereas the parent NSAID or aspirin had little or no effect(50,51). In these studies the anti-restenotic effect was correlated with reductions in vascular proliferation in the injured segment, and with plasma levels of nitrogen oxides. Another advantage of attaching an NO moiety to the NSAID or aspirin was a reduction in gastric ulceration, presumably due to cytoprotective effects and/or increased gastric blood flow produced by the NO released in the stomach. In one of these studies, the NO-releasing aspirin derivative (NCX-4016) reduced the degree of restenosis after balloon angioplasty in low-density lipoprotein receptor-deficient mice. This effect was associated with reduced vascular smooth muscle cell (VSMC) proliferation and macrophage deposition at the site of injury (51).

Local administration of NO donors or the NO precurson L-arginine has also been accomplished using channeled balloon catheters that permit the vessel wall to be bathed

by the agent during angioplasty. In the balloon injury model, vessels are denuded of endothelium, so that endothelial generation of NO from L-arginine is not possible initially. However, in the injured vessel wall, NO is produced by other cells such as proliferating vascular smooth muscle cells and infiltrating monocytes. Here, inducible NO synthase is responsible for NO production and L-arginine becomes rate-limiting(52). Indeed, it has been found that smooth muscle cells in the neointima express inducible NO synthase as early as 1 day after balloon catheter injury and this expression persists for up to 14 days(53). The local expression of iNOS inhibits platelet adherence and aggregation at the injured site; indeed systemic or adventitial application of NOS antagonists increases platelet adhesion at the injured site(54).

Notably, a single intramural administration of an NO donor or the NO precursor can have long-lasting effects on vascular structure and reactivity after balloon angioplasty. A single intraluminal administration of L-arginine has been shown to cause a sustained enhancement of vascular NO generation in the injured segment, resulting in improved vasomotion and inhibition of lesion formation(55). In a subsequent study, hypercholesterolemic New Zealand White rabbits underwent iliac artery angioplasty and a local drug delivery catheter was introduced into both iliac arteries to deliver either L-arginine (800 mg/5 mL) or saline(56). Intramural administration of radioactively labeled L-arginine led to significantly higher counts in comparison to the contralateral segment for up to 1 week after delivery; this was associated with significantly higher NO levels in the L-arginine–treated segments. For a prolonged period after this single administration(2-4 weeks), monocyte binding to the injured segment was significantly decreased by treatment with L-arginine, and there was a 9-fold greater number of apoptotic cells in the vessel wall.

Similar effects have been achieved with local delivery of NO donors. Rolland et al investigated the therapeutic effect of angioplasty with local drug delivery (LDD) of the NO-donor molsidomine in the superficial femoral arteries of atherosclerotic swine(57). Atherosclerotic Pietrin swine underwent angioplasty with delivery of 4 mg molsidomine in 2 ml of vehicle using a channelled balloon angioplasty catheter. In comparison to vehicle control, 24 hours after the injury, there was about a 60% reduction in proliferating vascular smooth muscle cells in the NO donor treated vascular segments as demonstrated by staining for PCNA-positive nuclei. At 5 months, molsidomine treated vessels, manifested increased compliance and reduced impedance. Histomorphometry revealed less restenotic intimal thickening and a greater lumenal diameter(by about 35%) in molsidomine-treated versus placebo-treated vessels.

Similar results were obtained by Kalinowski et al(58). New Zealand white rabbits underwent balloon dilation of both common iliac arteries to induce arterial stenosis. Four weeks later, one stenotic iliac artery was simultaneously dilated and received local application of L-arginine (210 mg/mL, n = 7), r-hirudin (0.5 mg/mL, n = 8), or molsidomine (0.2 mg/mL, n = 8) with a channeled balloon catheter. On the contralateral side, 0.9% saline was injected as a control. Six weeks after local treatment, vessels were harvested, and computerized morphometric and immunohistologic analyses were performed. In comparison to vehicle treated segments, those treated with L-arginine,

molsidomine or hirudin manifested significant reductions in myointimal hyperplasia (53%, 43%, and 20% respectively) in comparison to control. Immunohistologic findings showed a significant reduction of macrophages and proliferating cells in the neointima after local application of L-arginine.

Similar findings have been observed with the S-nitrosothiol class of NO donors. In one study, S-nitroso-bovine serum albumin, or a polythiolated form of bovine serum albumin modified to carry several S-nitrosothiol groups, were administered intraluminally to the injured rabbit femoral artery. The single administration of the nitrosylated peptides increased vascular NO generation, increased tissue cGMP, inhibited platelet aggregation and deposition at the site of injury, and significantly reduced myointimal hyperplasia assessed at 2 weeks following the injury(59). These effects were directly related to the amount of NO released at the site of vascular injury, with the polythiolated form being more efficacious.

The diazeniumdiolated derivative of albumin (D-BSA), is a derivatized protein containing 22 diazeniumdiolate groups per molecule with a 20-day half-life for NO release. Intrapericardial administration of D-BSA reduced coronary artery myointimal hyperplasia at two weeks by 50% in comparison to underivatized albumin in a swine model of balloon angioplasty(60). In addition, positive remodeling was noted, with a larger total vascular cross-sectional area. There was no systemic effect of this regional application of NO-donor. Specifically, the regional administration of an NO donor did not affect heart rate or blood pressure. Nor were any histological changes noted in the pericardium or myocardium(60). Similarly, periadventitial exposure of rat iliofemoral arteries to a gel containing an NO-releasing diazeniumdiolate during and after balloon injury produced a marked reduction of intimal hyperplasia 2 weeks after vascular injury(61).

Incorporation of NO donors into polymers may be useful for device applications. Nitric oxide donors with different half-lives have been covalently incorporated into photopolymerized polyethylene glycol hydrogels(62). Under physiological conditions, NO was produced by these hydrogels over periods ranging from hours to months, depending upon the polymer formulation. The NO-releasing materials successfully inhibited smooth muscle cell growth in culture. Platelet adhesion to collagen-coated surfaces was also inhibited following exposure of whole blood to NO-producing hydrogels.

To assess the effect of a NO-eluting stent on reducing neointimal thickening in a porcine coronary artery stent injury model, sodium nitroprusside (SNP), a NO donor, was incorporated into polyurethane polymer and coated onto metallic coil stents, and two types of stents with thin and thick barrier coatings were characterized. In vitro studies revealed that the SNP-coated stents released NO in a controlled manner for up to 4 weeks. In the in vivo studies, an increase in vascular cGMP levels at the site of the stent implantation was demonstrated for up to 14 days. The neointimal area at 28 days was not diminished, however, by NO eluting stents. The lack of an effect may have been due to inadequate tissue levels, or insufficient duration of release. (63). Similar results were

obtained using a tantalum coil coronary stents covered with an NO donor(64).

Clinical Investigations into NO and Restenosis

An intriguing study by Fukomoto and colleagues indicated that the ability of the vascular wall to produce NO after angioplasty correlated with less risk for restenosis(65). In 23 consecutive patients, the ability of the vessel wall to vasodilate in response to intra-coronary L-arginine infusion was assessed 18 hours after angioplasty (at a point in time were iNOS should be induced locally at the site of the vascular injury). L-arginine infusion induced a greater vasodilation at the angioplasty site, than at a distal uninjured segment. Notably, the magnitude of the vasodilator response to L-arginine correlated with the coronary artery diameter 3 months after PTCA. These results suggest that augmented NO production after PTCA may protect against the development of coronary restenosis. The authors surmised that "treatment that enhances local NO production may be clinically useful in preventing restenosis after PTCA".

Further proof of concept was obtained in the ACCORD study(66). This was a prospective multicenter, randomized trial, in which 700 stable coronary patients scheduled for angioplasty received direct NO donors (infusion of linsidomine followed by oral molsidomine) or oral diltiazem. Treatment was started before angioplasty and continued until 12 to 24 hours before follow-up angiography at 6 months. Pretreatment with an NO donor was associated with a modest improvement in the immediate angiographic result compared with pretreatment with diltiazem (minimum luminal diameter, 1.94 versus 1.81 mm; P = .001); this improvement was maintained at the 6-month angiographic follow-up (minimal lumen diameter, 1.54 versus 1.38 mm; P = .007). Restenosis, defined as a binary variable (> or = 50% stenosis), occurred less often in the NO donor group (38.0% versus 46.5%; P = .026). Combined major clinical events (death, nonfatal myocardial infarction, and coronary revascularization) were similar in the two groups (32.2% versus 32.4%). The investigators concluded that treatment with the NO donor was associated with a modest improvement in the long-term angiographic result after angioplasty although there was no effect on clinical outcome. The improved angiographic result related predominantly to a better immediate procedural result, because late luminal loss did not differ significantly between groups. The modest effects of NO donors on restenosis in this trial may be due to the fact that the NO donor was administered systemically, rather than at high local concentrations.

Support for this view was provided by a recent study examining the effect of intramural administration of L-arginine on in-stent restenosis(67). To determine whether intramural administration of L-arginine reduces intimal thickening after coronary stent deployment in humans, 50 patients with native coronary artery disease who received a single Palmaz-Schatz stent were enrolled in this pilot study. Patients were randomized to receive L-arginine (600 mg/6 ml) or saline (6 ml) delivered locally via the Dispatch catheter (Scimed) over 15 minutes. Serial angiography and intravascular ultrasound examinations

(motorized pull-back at 0.5 mm/s) were performed before and after the procedure, and at 6-month follow-up. At 6 months, neointimal volume in the L-arginine group was reduced by 36%.

Platelets are activated in patients undergoing PTCA as demonstrated by measurement of surface expression of P-selectin and glycoprotein IIb/IIIa in the platelets derived from coronary sinus vein blood samples despite systemic treatment with aspirin, glyceryl trinitrate, and heparin. Intracoronary infusion of the NO donor GSNO, starting 10 min before PTCA, significantly inhibited the PTCA-induced increase in platelet surface expression of P-selectin and glycoprotein IIb/IIIa without altering blood pressure(68). Thus local administration of NO donors can also inhibit platelet activation that occurs in the setting of angioplasty. This evidence is pre-clinical studies, local administration of NO donors to the injured vessel wall should also reduce acute thrombosis after angioplasty and stenting.

Summary

Nitric oxide is a potent vasodilator, and has significant effects on vascular structure by virtue of its ability to suppress vascular smooth muscle proliferation, reduce platelet adherence and aggregation, inhibit leukocyte infiltration, increase apoptosis of proliferating and inflammatory cells, and enhance endothelial regeneration. Local enhancement of vascular NO activity at the site of vascular injury may be an alternative therapeutic strategy to inhibit thrombosis and restenosis after balloon angioplasty and stenting.

References

1.) Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980 Nov 27;288(5789):373-6.

2.) Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical.
Circ Res. 1987 Dec;61(6):866-79.

3.) Murad F. The 1996 Albert Lasker Medical Research Awards. Signal transduction using nitric oxide and cyclic guanosine monophosphate.
JAMA. 1996 Oct 9;276(14):1189-92.

4.) Michel T, Feron O. Nitric oxide synthases: which, where, how, and why?
J Clin Invest. 1997 Nov 1;100(9):2146-52.

5.) Cooke JP, Dzau VJ: Nitric oxide synthase: Role in the genesis of vascular disease. *Annu Rev Med* 1997; 48:489-509

6.) Cooke JP, Tsao PS: Is NO an endogenous anti-atherogenic molecule? *Arterioscler Thromb* 1994; 14:653-655

7.) Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME: Anti-atherogenic effects of L-arginine in the hypercholesterolemic rabbit. *J Clin Invest* 1992 Sep;90(3):1168-72

8.) Aji W, Ravalli S, Szabolcs M, Jiang XC, Sciacca RR, Michler RE, et al. L-arginine prevents xanthoma development and inhibits atherosclerosis in LDL receptor knockout mice. Circulation 1997;95:430–437.

9.) Böger RH, Bode-Böger SM, Brandes RP, Phivthong-ngam L, Bohme M, Nafe R, et al. Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: comparison with lova-statin. Circulation 1997;96:1282–1290.

10.) McNamara DB, Bedi B, Aurora H, Tena L, Ignarro LJ, Kadowitz PJ, Akers DL. L-arginine inhibits balloon catheter-induced intimal hyperplasia. Biochem Biophys Res Commun. 1993 May 28;193(1):291-6.

11.) Holm AM, Andersen CB, Haunsø S, et al. Effects of L-arginine on vascular smooth muscle cell proliferation and apoptosis after balloon injury. *Scand Cardiovasc J.* 2000; 34: 28–32

12.) Wu Z, Qian X, Cai D, Liu Y, Li Z, He J. Long-term oral administration of L-arginine enhances endothelium-dependent vasorelaxation and inhibits neointimal thickening after endothelial denudation in rats. Chin Med J (Engl). 1996 Aug;109(8):592-8.

13.) von der Leyen HE, Gibbons GH, Morishita R, Lewis NP, Zhang L, Nakajima M, Kaneda Y, Cooke JP, Dzau VJ: Gene therapy inhibiting neointimal vascular lesion: *In vivo* transfer of endothelial cell nitric oxide synthase gene. *Proc Natl Acad Sci USA* 1995 Feb 14;92(4):1137-41

14.) Janssens S, Flaherty D, Nong Z, Varenne O, van Pelt N, Haustermans C, Zoldhelyi P, Gerard R, Collen D. Human endothelial nitric oxide synthase gene transfer inhibits vascular smooth muscle cell proliferation and neointima formation after balloon injury in rats. Circulation. 1998 Apr 7;97(13):1274-81.

15.) Shears LL II, Kibbe MR, Murdock AD, et al. Efficient inhibition of intimal hyperplasia by adenovirus-mediated inducible nitric oxide synthase gene transfer to rats and pigs in vivo. *J Am Coll Surg.* 1998; 187: 295–306

16.) Varenne O, Pislaru S, Gillijns H, Van Pelt N, Gerard RD, Zoldhelyi P, Van de Werf F, Collen D, Janssens SP. Local adenovirus-mediated transfer of human endothelial nitric oxide synthase reduces luminal narrowing after coronary angioplasty in pigs. Circulation. 1998 Sep 1;98(9):919-26.

17.) Cayatte AJ, Palacino JJ, Horten K, Cohen RA. Chronic inhibition of nitric oxide production accelerates neointima formation and impairs endothelial

function in hypercholesterolemic rabbits.
Arterioscler Thromb. 1994 May;14(5):753-9.

18.) Kuhlencordt PJ, Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, Hajjar R, Picard MH, Huang PL. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. Circulation. 2001 Jul 24;104(4):448-54.

19.) Le Tourneau, T. , Van Belle, E. , Corseaux, D. , Vallet, B. , Lebuffe, G. , Dupuis, B. , Lablanche, J. M. , McFadden, E. , Bauters, C. & Bertrand, M. E. (1999) *J. Am. Coll. Cardiol.* 33, 876-882

20.) Loscalzo J. Antiplatelet and antithrombotic effects of organic nitrates. Am J Cardiol. 1992 Sep 24;70(8):18B-22B

21.) Mellion BT, Ignarro LJ, Ohlstein EH, Pontecorvo EG, Hyman AL, Kadowitz PJ. Evidence for the inhibitory role of guanosine 3', 5'-monophosphate in ADP-induced human platelet aggregation in the presence of nitric oxide and related vasodilators. Blood. 1981 May;57(5):946-55.

22.) Butt E, Abel K, Krieger M, Palm D, Hoppe V, Hoppe J, Walter U. cAMP- and cGMP-dependent protein kinase phosphorylation sites of the focal adhesion vasodilator-stimulated phosphoprotein (VASP) in vitro and in intact human platelets. J Biol Chem. 1994 May 20;269(20):14509-17.

23.) Aszodi A, Pfeifer A, Ahmad M, Glauner M, Zhou XH, Ny L, Andersson KE, Kehrel B, Offermanns S, Fassler R. The vasodilator-stimulated phosphoprotein (VASP) is involved in cGMP- and cAMP-mediated inhibition of agonist-induced platelet aggregation, but is dispensable for smooth muscle function. *EMBO J.* 1999;18:37–48

24.) Jawien A, Bowen-Pope DF, Lindner V, Schwartz SM, Clowes AW. Platelet-derived growth factor promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. J Clin Invest. 1992 Feb;89(2):507-11.

25.) Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci U S A. 1991 Jun 1;88(11):4651-5.

26.) Tsao PS, Wang B, Buitrago R, Shyy JY, Cooke JP. Nitric oxide regulates monocyte chemotactic protein-1. *Circulation.* 1997;96:934–940

27.) Tsao PS, Buitrago R, Chan JR, Cooke JP. Fluid flow inhibits endothelial adhesiveness. Nitric oxide and transcriptional replication of VCAM-1. *Circulation.* 1996;94:1682–1689

28.) Zeiher AM, Fisslthaler B, Schray-Utz B, Busse R. Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. Circ Res. 1995 Jun;76(6):980-6.

29.) Peng HB, Libby P, Liao JK. Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. J Biol Chem 1995;270:14,214–14,219.

30.) Clancy RM, Leszczynska P, Piziak J, Abramson SB. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on NADPH oxidase. *J Clin Invest.* 1992;90:1116–1121

31.) Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromocyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest.* 1989;83:1774–1777

32.) Sarkar R, Gordon D, Stanley JC, Webb RC. Dual cell cycle-specific mechanisms mediate the antimitogenic effects of nitric oxide in vascular smooth muscle cells. J Hypertens. 1997 Mar;15(3):275-83.

33.) Sato J, Nair K, Hiddinga J, Eberhardt NL, Fitzpatrick LA, Katusic ZS, O'Brien T. eNOS gene transfer to vascular smooth muscle cells inhibits cell proliferation via upregulation of p27 and p21 and not apoptosis. Cardiovasc Res. 2000 Sep;47(4):697-706.

34.) Mooradian DL, Hutsell TC, Keefer LK. Nitric oxide (NO) donor molecules: effect of NO release rate on vascular smooth muscle cell proliferation in vitro. *J Cardiovasc Pharmacol.* 1995; 25: 674–678

35.) Kahn AM, Allen JC, Seidel CL, Zhang S. Insulin inhibits migration of vascular smooth muscle cells with inducible nitric oxide synthase. Hypertension. 2000 Jan;35(1 Pt 2):303-6.

36.) Brown C, Pan X, Hassid A. Nitric oxide and C-type atrial natriuretic peptide stimulate primary aortic smooth muscle cell migration via a cGMP-dependent mechanism: relationship to microfilament dissociation and altered cell morphology. Circ Res. 1999 Apr 2;84(6):655-67.

37.) Pollman MJ, Yamada T, Horiuchi M, Gibbons GH. Vasoactive substances regulate vascular smooth muscle cell apoptosis. Countervailing influences of nitric oxide and angiotensin II. *Circ Res.* 1996;79:748–756

38.) Dimmeler S, Haendeler SJ, Nehls M, Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1ß-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. *J Exp Med.* 1997;185:601–607

39.) Bennett MR, Evan GI, Schwarz SM. Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. *J Clin Invest.* 1995;95:2266–2274

40.) Isner JM, Kearney M, Bortman S, Passeri J. Apoptosis in human atherosclerosis and restenosis. *Circulation.* 1995;91:2703–2711

41.) Geng YJ, Wu Q, Muszynski M, Hansson GK, Libby P. Apoptosis of vascular smooth muscle cells induced by in vitro stimulation with interferon-gamma, tumor necrosis factor-alpha, and interleukin-1beta. *Arterioscler Thromb Vasc Biol.* 1996;16:19–27

42.) Buttery LD, Speringall DR, Chester AH, Evans TJ, Standfield EN, Parums DV, Yacoub MH, Polak JM. Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. *Lab Invest.* 1996;75:77–85.

43.) Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, the ugly. *Am J Physiol.* 1996;271:C1424–1437.

44.) Nguyen TBD, Crepsi CL, Penman BW, Wishnok JS, Tannenbaum SR. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci U S A.* 1992;89:3030–3034

45.) Wang BY, Candipan RC, Arjomandi M, Hsiun PT, Tsao PS, Cooke JP. Arginine restores nitric oxide activity and inhibits monocyte accumulation after vascular injury in hypercholesterolemic rabbits. *J Am Coll Cardiol.* 1996;28:1573–1579.

46.) Janero DR, Ewing JF. Nitric oxide and postangioplasty restenosis: pathological correlates and therapeutic potential. *Free Radic Biol Med.* 2000; 29: 1199–1221

47.) Provost P, Tremblay J, Merhi Y. The antiadhesive and antithrombotic effects of the nitric oxide donor SIN-1 are combined with a decreased vasoconstriction in a porcine model of balloon angioplasty. *Arterioscler Thromb Vasc Biol.* 1997; 17: 1806–1812

48.) Guo J, Milhoan KA, Tuan RS, et al. Beneficial effect of SPM-5185, a cysteine-containing nitric oxide donor, in rat carotid artery intimal injury. *Circ Res.* 1994; 75: 77–84) .

49.) Lee JS, Adrie C, Jacob HJ, Roberts JD Jr, Zapol WM, Bloch KD. Chronic inhalation of nitric oxide inhibits neointimal formation after balloon-induced arterial injury. Circ Res. 1996 Feb;78(2):337-42.

50.) Maffia P, Ianaro A, Sorrentino R, Lippolis L, Maiello FM, del Soldato P, Ialenti A, Cirino G. Beneficial effects of NO-releasing derivative of flurbiprofen (HCT-1026) in rat model of vascular injury and restenosis. Arterioscler Thromb Vasc Biol. 2002 Feb 1;22(2):263-7.

51.) Napoli C, Aldini G, Wallace JL, de Nigris F, Maffei R, Abete P, Bonaduce D, Condorelli G, Rengo F, Sica V, D'Armiento FP, Mignogna C, de Rosa G, Condorelli M, Lerman LO, Ignarro LJ. Efficacy and age-related effects of nitric oxide-releasing aspirin on experimental restenosis. Proc Natl Acad Sci U S A. 2002 Feb 5;99(3):1689-94.

52.) Joly GA, Schini VB, Vanhoutte PM. Balloon injury and interleukin-1ß induce nitric oxide synthase activity in rat carotid arteries. *Circ Res.* 1992;71:331–338.

53.) Hansson GK, Geng YJ, Holm J, Hardhammar P, Wennmalm A, Jennische E. Arterial smooth muscle cells express nitric oxide synthase in response to endothelial injury. *J Exp Med.* 1994;180:733–738

54.) Yan ZQ, Yokota T, Zhang W, Hansson GK. Expression of inducible nitric oxide synthase inhibits platelet adhesion and restores blood flow in the injured artery. Circ Res. 1996 Jul;79(1):38-44.

55.) Schwarzacher SP, Lim TT, Wang BY, Kernoff RS, Niebauer J, Cooke JP, Yeung AC. Local intramural delivery of L-arginine enhances nitric oxide generation and inhibits lesion formation after balloon angioplasty. *Circulation.* 1997;95:1863–1869.

56.) Niebauer J, Schwarzacher SP, Hayase M, Wang B, Kernoff RS, Cooke JP, Yeung AC: Local L-arginine delivery after balloon angioplasty reduces monocyte binding and induces apoptosis. *Circulation* 1999 Oct 26;100(17): 2830-5

57.) Rolland PH, Bartoli JM, Piquet P, Mekkaoui C, Nott SH, Moulin G, Amabile P, Mesana T. Local delivery of NO-donor molsidomine post-PTA improves haemodynamics, wall mechanics and histomorphometry in atherosclerotic porcine SFA. Eur J Vasc Endovasc Surg. 2002 Mar;23(3):226-33.

58.) Kalinowski M, Alfke H, Bergen S, Klose KJ, Barry JJ, Wagner HJ. Comparative trial of local pharmacotherapy with L-arginine, r-hirudin, and molsidomine to reduce restenosis after balloon angioplasty of stenotic rabbit iliac arteries. Radiology. 2001 Jun;219(3):716-23.

59.) Marks DS, Vita JA, Folts JD, Keaney JF Jr, Welch GN, Loscalzo J. Inhibition of neointimal proliferation in rabbits after vascular injury by a single treatment with a protein adduct of nitric oxide. J Clin Invest. 1995 Dec;96(6):2630-8.

60.) Baek SH, Hrabie JA, Keefer LK, Hou D, Fineberg N, Rhoades R, March KL. Augmentation of intrapericardial nitric oxide level by a prolonged-release nitric oxide donor reduces luminal narrowing after porcine coronary angioplasty. Circulation. 2002 Jun 11;105(23):2779-84.

61.) Kaul S, Cercek B, Rengstrom J, et al. Polymeric-based perivascular delivery of a nitric oxide donor inhibits intimal thickening after balloon denudation arterial injury: role of nuclear factor-kappaB. *J Am Coll Cardiol.* 2000; 35: 493–501).

62.) Bohl KS, West JL. Nitric oxide-generating polymers reduce platelet adhesion and smooth muscle cell proliferation. Biomaterials. 2000 Nov;21(22):2273-8.

63.) Yoon JH, Wu CJ, Homme J, Tuch RJ, Wolff RG, Topol EJ, Lincoff AM. Local delivery of nitric oxide from an eluting stent to inhibit neointimal thickening in a porcine coronary injury model. Yonsei Med J 2002 Apr;43(2):242-51

64.) Buergler JM, Tio FO, Schulz DG, Khan MM, Mazur W, French BA, Raizner AE, Ali NM. Use of nitric-oxide-eluting polymer-coated coronary stents for prevention of restenosis in pigs. Coron Artery Dis. 2000 Jun;11(4):351-7.

65.) Fukumoto Y, Urabe Y, Kubo T, Kaku T, Egashira K, Shimokawa H, Takeshita A. Heart Vessels 2002 Jul;16(5):171-174

66.) Lablanche JM, Grollier G, Lusson JR, Bassand JP, Drobinski G, Bertrand B, Battaglia S, Desveaux B, Juilliere Y, Juliard JM, Metzger JP, Coste P, Quiret JC, Dubois-Rande JL, Crochet PD, Letac B, Boschat J, Virot P, Finet G, Le Breton H, Livarek B, Leclercq F, Beard T, Giraud T, Bertrand ME, et al. Effect of the direct nitric oxide donors linsidomine and molsidomine on angiographic restenosis after coronary balloon angioplasty. The ACCORD Study. Angioplastic Coronaire Corvasal Diltiazem. Circulation. 1997 Jan 7;95(1):83-9.

67.) Suzuki T, Hayase M, Hibi K, Hosokawa H, Yokoya K, Fitzgerald PJ, Yock PG, Cooke JP, Suzuki T, Yeung AC. Effect of local delivery of L-arginine on in-stent restenosis in humans. Am J Cardiol. 2002 Feb 15;89(4):363-7.

68.) Langford EJ, Brown AS, Wainwright RJ, de Belder AJ, Thomas MR, Smith RE, Radomski MW, Martin JF, Moncada. Inhibition of platelet activity by S-nitrosoglutathione during coronary angioplasty. Lancet. 1994 Nov 26;344(8935):1458-60.